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Microcalorimetric investigations on isolated tumorous and non-tumorous tissue samples [1]

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Abstract

In this study, microcalorimetric measurements were carried out on 44 tumorous and non-tumorous tissue samples from organs of the uro-genital tract as well as from the mammae and the colon. The aim of this investigation was to compare the results of the microcalorimetric measurements with those of impulse-cytophotometrical and histological examinations in order to provide evidence of the metabolic activity of tumorous and non-tumorous tissues. The course of the heat output of the tissue samples was measured at one-minute intervals and graphically displayed as a function of time. For the evaluation of the results of the microcalorimetrically measured values, the maxima (P_{max}), the mean values ($\bar{P}$), and the contour integrals (W) of the microcalorimetric curves were determined. It was found that, without exception, all measured metabolic activity values of tumorous tissue samples were distinctly higher than those of non-tumorous tissue samples from the same organ. We conclude that through a microcalorimetric analysis, it is possible to differentiate between healthy and tumorous tissue samples on the basis of a varyingly higher metabolic activity of the malignant samples.

INTRODUCTION

The standard and reference methods for determining the malignancy level of a tumor are the histological examination and the automatic measurement of the DNA content of the cell nuclei by impulse-cytophotometry (ICP) [1, 2]. With these procedures, the established degrees of differentiation of the tissue sample under investigation describes the malignancy level of the tumor, which is a decisive factor in the prognosis and therapy [3]. One procedure that can determine the "biological activity" of a living system, e.g. a tissue sample, is available in the form of direct calorimetry. It provides a continuous measurement of the heat production,

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and thereby gives information about the activity of the total metabolism of the tissue sample under examination. In 1986, the first calorimetric measurement of the metabolic activity of tumor cells was carried out by a Swedish team [4]. These measurements showed a correlation between the metabolic activity of the tumor cells and the survival rate of the patient. Such findings lead to the question of whether the measurement of the "biological activity" of a tissue sample could be used to draw a parameter of malignancy which perhaps could give more information on the prognosis and dynamic behavior of the tumor than purely morphological methods [5–7]. The aim of this study, therefore, was to determine if reproducible differences between tumorous and non-tumorous tissues could be obtained by means of microcalorimetry.

METHOD

A total of 44 tissue samples from uro-genital tract organs as well as from the mammae and the colon were available for microcalorimetric measurements using a Thermal Activity Monitor (TAM). The samples originated from patients in the urological, gynecological and surgical units at the University clinics of Göttingen, requiring biopsy for therapeutic or diagnostic reasons. After the tissue samples had been transported to the calorimetric laboratory under hypothermic conditions, i.e. at 4°C, they were separated into three parts (Fig. 1). One part was to be examined histologically, the second cytochemically, i.e. with the aid of impulse-cytophotometry, and the third microcalorimetrically. Through the use of the established reference methods, it was clear that the tissue samples from the bladder, the prostate, the kidney, the adrenal glands, the testicles, the uterus, the mammae and the colon comprised non-tumorous and tumorous material at all stages. For the microcalorimetric examination of the tissue samples, glass ampoules filled with 2.5 ml of Ringer solution were used. The

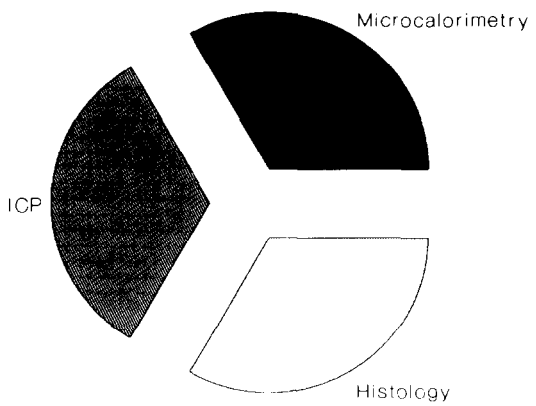

Fig. 1. The different techniques applied to the analysis of tissue samples: ICP, impulse-cytophotometry.

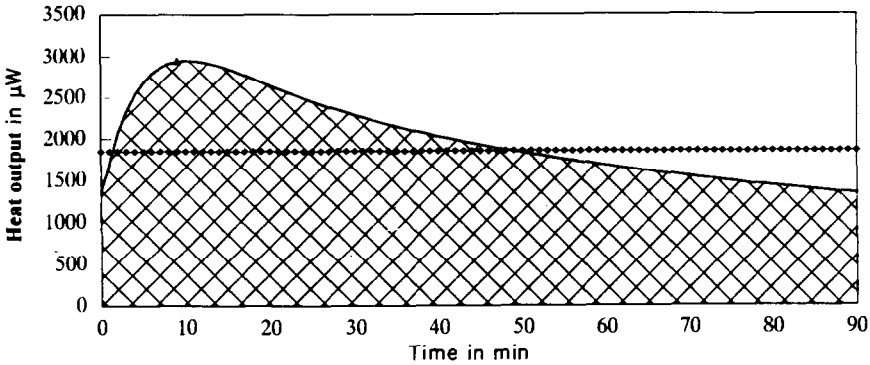

Fig. 2. Parameters used to analyse microcalorimetric records obtained from tissue samples: shaded area, the total energy released W; horizontal line, mean thermal power $\bar{P}$.

measurements were carried out at 37°C corresponding to the physiological body temperature, for a 90 min time period. Then, the samples were dried at 100°C until weight constancy. Finally, the measured values were converted into microwatts per gram dry weight (dw). With the static ampoules conditions used in this study, typically decaying calorimetric curves were obtained, indicating the successive loss of metabolic activity of the non-perfused tissue samples.

For the evaluation of these heat flow–time curves, three parameters were used: the maximal thermal power (P_{max} in μW per g dw); the integral of the curve serving as a measure of the total energy released (W in mJ per g dw); and the mean thermal power ($\bar{P}$ in μW per g dw) of the tissue sample during the period of measurement (see Fig. 2).

For a direct comparison between tumorous and non-tumorous mammae tissue, only the P_{max} values are available, because the measurement of non-tumorous mammae tissue was interrupted after a 60 min period.

RESULTS

Taking these parameters into consideration, the tumorous tissue samples generally showed a higher metabolic activity than the non-tumorous specimens. For instance, in bladder tissues, tumorous samples clearly exhibited, from the beginning and also during the course of the measurement, a distinctly higher metabolic activity than the non-tumorous tissue samples of this organ. The metabolic activity of the tumorous material was higher by a factor of 2.5–3.1 than the non-tumorous samples (Fig. 3).

For the 13 measurements on prostate tissues, the tumorous samples also showed a distinctly higher activity than the non-tumorous tissues. The total released energy W during the measurement period and the mean thermal power $\bar{P}$ were greater for tumorous tissue samples by a factor of 8, and likewise, the maximum thermal power P_{max} was increased by a factor of 8.4 (Fig. 4).

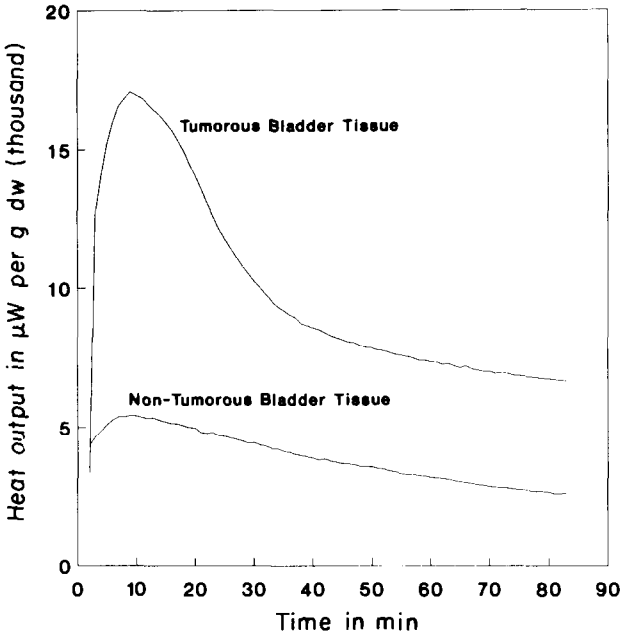

Fig. 3. Microcalorimetric investigations on bladder tissues.

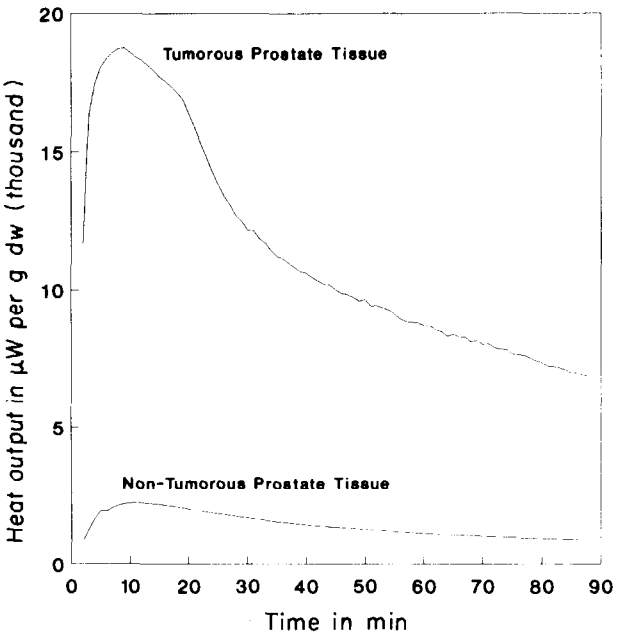

Fig. 4. Microcalorimetric investigations on prostate tissues.

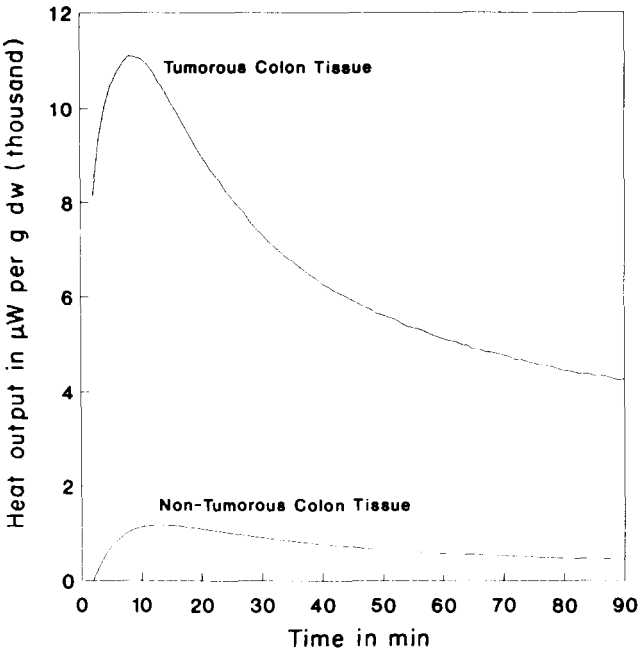

Fig. 5. Microcalorimetric investigations on colon tissues.

Measurements of the colon tissue samples also yielded a distinctly higher metabolic activity for tumorous samples in comparison to non-tumorous samples: all of the parameters in question, P_{max}, W and $\bar{P}$, were increased by more than a factor of 9 in the tumorous samples (Fig. 5).

For the assessment of the metabolic activity of kidney tissues, a total of 11 tissue samples from six patients were available. As the thermograms clearly show (Fig. 6), the tumorous kidney tissue also manifests a higher metabolic activity by a factor of 1.4–1.8 than non-tumorous tissue. However, the lowest metabolic activity was recorded in predominantly necrotic parts of kidney cell carcinoma. Therefore, a methodological problem in tissue calorimetry is revealed. Despite the fact that this result derives from tumorous material, the measured metabolic activity is distinctly lower than in the non-tumorous tissue. A reason for this non-conformity is that a large number of non-vital cells contribute to a considerably reduced metabolism. These results show that a very accurate, careful histological examination and "vitality control" of the samples cannot be dispensed with. Figures 7–9 show a direct comparison, for each parameter, between tumorous and non-tumorous samples of the tissues investigated. Thus it can be seen that, without exception, the metabolic activity in the tumorous samples is higher than in the non-tumorous samples of the same tissue. Therefore, a differentiation between malignant and benign tissues (regardless of which of the three parameters is considered) is possible through their differing metabolic activities.

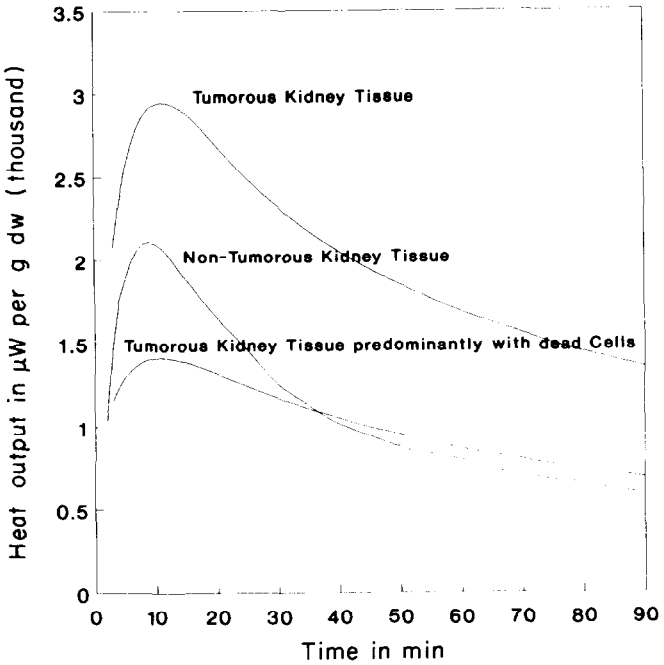

Fig. 6. Microcalorimetric investigations on kidney tissues.

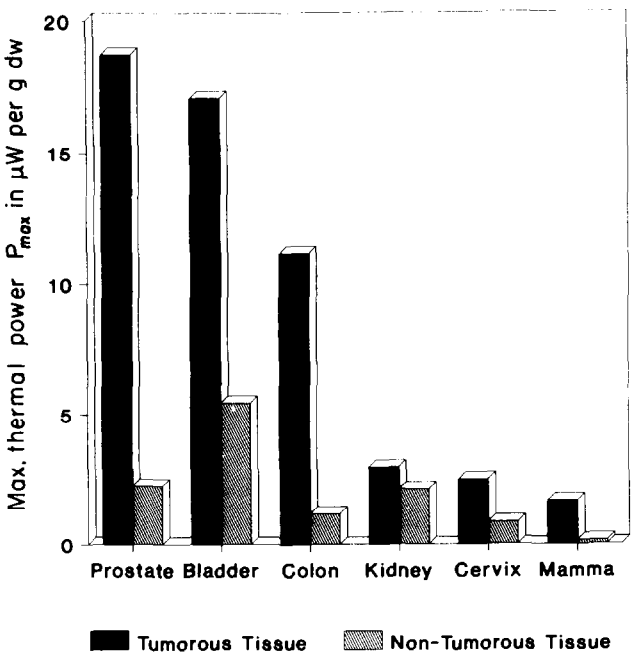

Fig. 7. Comparison of the maximal thermal power (P_{max}) values of all tissue samples.

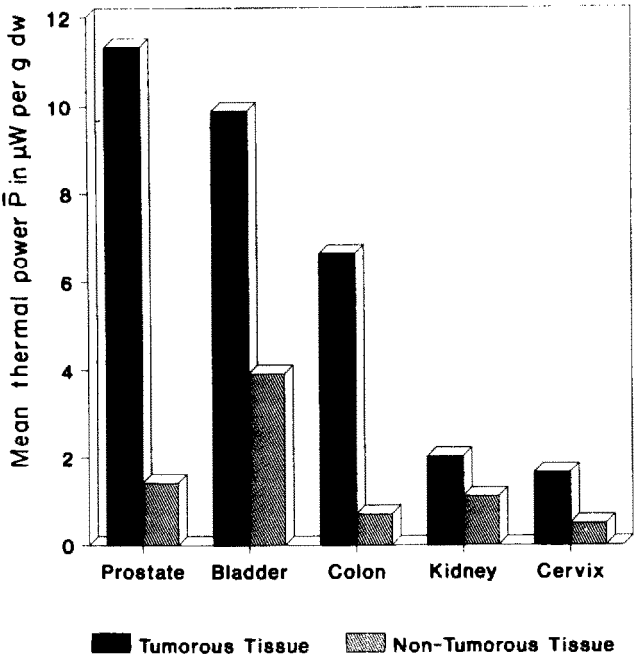

Fig. 8. Comparison of the mean thermal power ($\bar{P}$) values of all tissue samples.

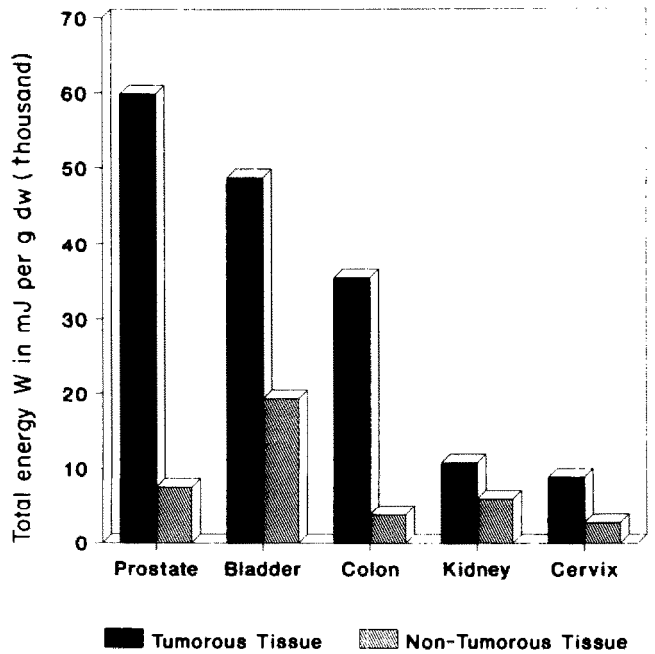

Fig. 9. Comparison of the contour integrals (W) of all tissue samples.

DISCUSSION

At first glance, these results are not surprising because earlier examinations [4, 8] had found a higher metabolic activity in tumorous samples than in non-tumorous material. In view of this increased metabolic activity of tumorous material, one would expect, however, a faster decay rate than in non-tumorous tissue under ischemic conditions, i.e. with a decrease in blood and substrate supply. Yet as the measurements have revealed, the opposite is the case. In corroboration of these results, the examinations carried out in 1957 by Opitz [9] are of interest: he investigated the percentage survival rate of various tissues with respect to the ischemic period (Fig. 10). Whereas the survival rate of brain tissue sinks to zero percent within a few minutes, a distinctly higher ischemia tolerance is ascertainable in kidney tissues. In these examinations, the highest survival rates were also shown by the carcinoma tissue.

This raises the question as to the causes that may be responsible for the delayed loss of metabolic activity in the tumorous samples as compared to the non-tumorous tissue samples. In the examinations performed by Warburg in 1930 [10], the results of which have been confirmed repeatedly ever since, an increase rate of anaerobic glycolysis is indicated in tumorous over non-tumorous tissues [11–15], which is partly due to an altered enzyme pattern in the neoplastic cells [16–18]. Because the survival of tissues under ischemic conditions is mainly dependent on anaerobic glycolysis, an increased anaerobic capacity would lead to a slower metabolic decay of tumorous as compared to non-tumorous samples [19] and might, hence, be a good explanation for the microcalorimetric findings presented in this study.

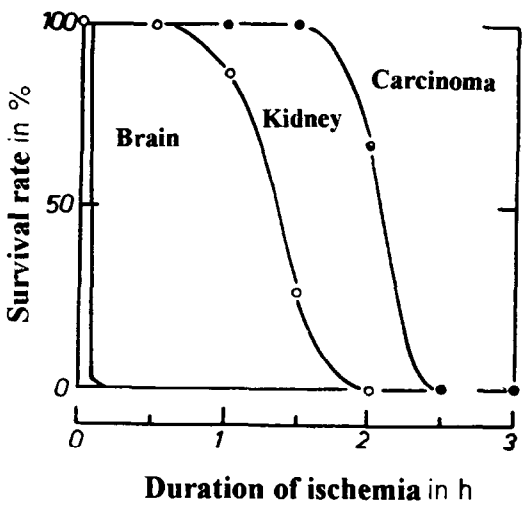

Fig. 10. Survival rate of different tissues under ischemic conditions (from ref. 9).

The metabolic difference between tumorous and non-tumorous material varied among the tissues investigated. The level of metabolic variation depended on the specific organ. For example, prostate, colon and bladder tissues had more distinctly higher metabolic differences between tumorous and non-tumorous samples than was the case for kidney tissues. Within the framework of this study, it could not be ascertained if a possible reason for varying metabolic activities was due to the different malignancy levels of tumors from the different organs. Further measurements are required in order to make definite conclusions about the metabolic behavior of different organ samples.

In conclusion, microcalorimetry as a means of measuring the "biological activities" of tissue samples could possibly provide additional information on tumor malignancy. Thus, after further standardization of the measurement procedure, which is an essential factor in obtaining reliable results, microcalorimetry could, in the long term, supplement the established methods of pre-operative tumor diagnosis and grading.

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